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Abstract

Aging research is undergoing a paradigm shift, which has led to new and innovative methods of exploring this complex phenomenon. The systems biology approach, endeavours to understand biological systems in a holistic manner, by taking account of intrinsic interactions, whilst also attempting to account for the impact of external inputs, such as diet. A key technique employed in

systems biology, is computational modeling, which involves mathematically describing and simulating the dynamics of biological systems. Although a large number of computational models have been developed in recent years, these models have focused on various discrete components of the aging process, and to date no model has succeeded in completely representing the full scope of aging. Combining existing models or developing new models may help to address this need and in so doing could help achieve an improved understanding of the intrinsic mechanisms which underpin aging.

INTRODUCTION- AGING AND THE NEED FOR COMPUTATIONAL SYSTEMS BIOLOGY

The world's population is aging. Globally, the number of older people (aged 60 years or over) is expected to more than double, from 841 million people in 2013 to more than 2 billion in 2050[1]. Those aged 80 years and over, the fastest growing group of older people, make up approximately 14% of the global population, and it is projected by 2050 there will be more than three times the present number of this age group. To help put this demographic shift into perspective, it is worth noting, that the number of older people in the world's population will exceed the number of younger people by 2047[1]. An aging population poses many challenges for all sectors of society. Particularly as advancing age is associated with an increased risk of developing many disease states, such as cancer[2], cardiovascular disease (CVD)[3], Alzheimer's disease (AD)[4] and Parkinson's disease[5]. Thus, there is a growing imperative to better understand the aging process and health-span. However, to date, there is no overall consensus as to what constitutes healthy-span[6] or what the key mechanisms are that underpin human aging. This is partly due to the inherent complexity of aging, which effects every component of a living system, from the disruption of DNA integrity to the dysregulation of whole-body homeostatic mechanisms (Figure 1)[7]. Thus, aging is especially challenging to investigate. Consequently there are many approaches to study the complexities of this phenomenon, from studying single genes in isolation, to using simple organisms such as yeast, or employing epidemiological studies. Over the last decade and half, aging research has become increasingly affected by the systems biology paradigm, which eschews reductionism and treats the organism as a whole[8, 9]. By placing aging research firmly within a systems biology framework a means of dealing with its intrinsic complexity is provided. A key element of this approach is the juxtapositioning of computational modelling with experimental investigations[10-12]. These models both compliment and inform the experimental work by facilitating hypothesis testing, generating new insights, deepening biological understanding, making predictions, tracing chains of causation, integrating knowledge, and inspiring new experimental approaches[13-15]. Computational models developed to date to understand the aging process, have in the main represented several discrete mechanisms that are associated with aging. Examples include models of mitochondrial dysregulation[16], telomere attrition[17] and the disruption of protein turnover[18]. Despite this, there are relatively few examples whereby aging has been represented using a computational model in a holistic fashion. In this paper we will 1) use oxidative stress as a framework to discuss the interconnectivity of aging 2) briefly outline the two main theoretical approaches used to assemble computational models in systems biology 3) discuss recent models that have been used to represent various aspects of aging 4) suggest how these models could be further developed in the future to lead to a more holistic representation of aging.

THE QUEST FOR A COMMON THREAD

Many theories have been proposed to explain the aging process. From an evolutionary standpoint aging is generally regarded as a non-adaptive process which is a by-product of evolution (for a review of the main evolutionary theories see Gavrilov and Gavrilova (2002)[19]). If we assume that aging is a by-product of evolution, the question remains, how does this process unfold? Moreover, is there a common thread that regulates aging in all organisms? It is generally accepted that aging is not underpinned by one biological mechanism, rather it is the result of the interaction between an array of processes that act over a diverse range of spatial and temporal scales. As a result of this consensus, it has been recognized that in order to gain a more complete understanding of the mechanics of aging, integration of multiple biological pathways need to be considered. However, despite this complexity, the free radical theory of aging is arguably the closest gerontology has come to a framework, which connects together the disparate aspects of the aging process. The free radical theory of aging proposes that damage to biological macromolecules by reactive oxygen species (ROS) accounts for aging[20]. Due to the role of the mitochondrial electron transport chain (ETC) in cellular respiration, mitochondria are central to this theory and are regarded as the main producers of ROS[21]. Together with other cellular organelles and macromolecules, mitochondria are vulnerable to the destructive capabilities of ROS. During aging mitochondrial DNA (mtDNA) accumulate deletions across a variety of somatic cell types[22, 23]. These deletions contribute to the overall decline in mitochondrial dysfunction[24]. Specifically, age-related mitochondrial changes include fusion and fission dysregulation[25], impaired proteostasis[26], diminished mitophagy[27] and diminished ATP production[28]. This damage to mitochondria affects their integrity, exacerbating ROS emissions and driving the aging process. This assertion is backed up by experimental evidence, which has shown that mitochondrial emission rates of $O_2^{-\cdot}$ and H_2O_2 increase continuously with age at species-specific rates [29]. In this paper we will use ROS as a conduit to emphasise the interconnected nature of the aging process and we will stress that no single factor is responsible for the aging process but rather a multitude of overlapping mechanisms. Moreover, it is imperative at this point, to emphasise that low levels of ROS have also been suggested to improve host resistance to oxidative damage in a process termed mitohormesis[30]. Thus, although it is generally regarded that ROS cause cellular damage, their role within the aging process maybe much broader.

Telomeres Attrition, Cellular Senescence and Oxidative Stress

The free radical theory of aging converges with a multitude of other cellular processes, which have been implicated with aging, including the maintenance of telomere integrity[31]. Telomeres are

repetitive TTAGGG sequences at the ends of chromosomes. Telomeres operate like a protective cap while telomerase, the enzyme responsible for maintaining telomere length, is largely absent from human somatic cells[32]. Consequently, each time a somatic cell divides, some of the telomere is lost. Hence, in humans, telomeres are shorter in older individuals. This was initially confirmed experimentally by the seminal work of Harley et al. (1990), who showed that both the quantity and length of telomeric DNA in human fibroblasts decrease during aging *in vitro*[33]. Moreover, the relationship between telomeres and cellular senescence was further cemented when telomerase-negative normal human cells were transfected with the telomerase catalytic subunit[34]. As a result, these cells had elongated telomeres, divided vigorously and displayed reduced senescence, when compared to telomerase-negative control clones, which exhibited telomere shortening and senescence[34]. More recently, investigations using telomerase knock-out rodents and human studies with telomere maintenance disorders have shown that a reduction in telomere length is associated with functional decline in a wide variety of tissues[35]. This brings us to oxidative stress and telomere shorting; experimental studies have determined that telomerase is not the sole factor governing the rate of loss of telomeric DNA. It has been shown that mild oxidative stress, as demonstrated by the culturing of human fibroblasts under 40% oxygen partial pressure, resulted in an increase telomere shortening from 90 base pairs(bp) per population doubling under normoxia, to more than 500 bp per population doubling under hyperoxia[36]. Thus, further embedding the free radical theory and oxidative stress as the epicentre of the aging process.

Caloric Restriction and Oxidative Stress

Oxidative stress is one possible mechanism which might explain the effect of caloric restriction (CR) on longevity. However, it is important to again stress at this point that oxidative damage is likely to be one key mechanism among many deleterious processes that underlie aging[37]. For instance, it is suggested that the beneficial effects of CR are mediated via a reduction in the production of ROS[36]. CR is a dietary regime that involves reducing nutrient intake without inducing malnutrition (usually a 20–40% reduction in calorie intake)[38]. CR has been demonstrated to extend lifespan in a diverse range of organisms[39-41]; although its effect on humans is yet to be fully established. What has been established is that CR positively effects mitochondrial function in a number of ways. Most notably, CR has been shown to reduce the emission of ROS. For example, CR dampens the release of ROS from complex I of mitochondria in cardiac tissue of rats[42]. Furthermore, it has also been found that CR lessens the accumulation of oxidative damage. This damage characterises aging, in many tissue types across a diverse array of species[43].

Sirtuins and Caloric Restriction

Metabolically, the effects of CR on the mitochondria could be modulated by several important biochemical pathways which have been implicated with increased longevity. For instance, in yeast mother cells the NAD+ dependent class III of histone deacetylase enzymes (sirtuins) have been suggested to mediate the life-extending effects of CR[44]. In particular sirtuin 2 (Sir2) is implicated in the response to CR in yeast models[45]. Homologues of Sir2 have been shown to mediate some of the effects of CR in other organisms. For instance, it has been reported that an increase in *Drosophila* Sir2 extends life span, whereas a decrease in Sir2 blocks the life-span-extending effect of CR[46], while similar findings have been reported in *Caenorhabditis elegans*[47]. Mammals possess 7 homologues of the Sir2 protein, which have been implicated in the regulation of a number of processes, from cell growth and apoptosis, to mitochondrial metabolism[48]. SIRT1, is the homologue of Sir2, a gene whose activity has also been shown to be modulated by CR[49]. For instance, it has been shown that expression of mammalian Sir2 (SIRT1) is induced in CR rats as well as in human cells that are treated with serum from these animals[50]. In certain cells this response could be induced by nitric oxide synthase (eNOS), which can activate the SIRT1 promoter[51]. This view is tentatively supported by recent findings from Shinmura *et al.* (2015), who showed that eNOS knock-out mice exhibited elevated blood pressure and left ventricular hypertrophy compared with wild-type mice, although they underwent CR[52]. Other sirtuins have also been implicated as mediators of the effects of CR[52]. For instance, mice lacking the mitochondrial deacetylase SIRT3 have been shown to suffer from increased levels of oxidative damage[53]. Specifically, this study showed that SIRT3 reduced cellular ROS levels by deacetylating superoxide dismutase 2 (SOD2), a major mitochondrial antioxidant enzyme. This alteration promoted its antioxidative activity, thus emphasising the close coupling of many of the factors that have been implicated in aging and longevity.

mTOR the Missing Metabolic Link?

Another key pathway implicated in longevity is the pathway defined by the mammalian target of rapamycin (mTOR)[54]. mTOR is a serine/threonine protein kinase of the phosphatidylinositol-3-OH kinase (PI(3)K)-related family. mTOR comprises of two separate complexes, mTORC1 and mTORC2, which coordinate a variety of nutrient and hormonal cellular signals, which control a variety of cellular processes including cell growth, cell size, and metabolism[55]. The connection between mTOR and longevity was first identified over two decades ago, when it was found that knocking out Sch9, the homolog of the mTORC1 substrate S6K, augmented chronological lifespan[56]. Subsequently, a number of key studies using a variety of organisms have revealed that the mTOR is highly conserved[57]. For example, mutations in daf-15 a homolog of Raptor, a constituent of mTORC1, can extend the lifespan of *C. elegans*. The mutants adapted their metabolism to accumulate lipids, while there was also an increase in adult life span[58]. Moreover, it has been suggested that the effects of CR are coordinated by mTOR. For instance, CR has been shown to activate eukaryotic translation initiation factor 4E-binding protein 1 in *Drosophila*[59]. Activation of this translation protein provoked an increase in the translation of several molecules involved in the mitochondrial electron transport chain and an increase in lifespan. This lifespan increase could be due to a concomitant drop in oxidative stress. This assertion is supported by experimental evidence, which has shown that the inhibition of mTORC1 lowers mitochondrial membrane potential, O_2 consumption and ATP levels[60]. In addition, mTOR has been shown to interact with other aspects mitochondrial function including biogenesis, apoptosis and mitochondrial hormesis[61].

Mitochondrial Function and Epigenetic Processes

Given the key role mitochondrial metabolism plays in ROS generation, and its putative connection with CR, it is worth considering how both mitochondrial function and the emission of ROS interact with other important biochemical and genetic processes. The Krebs cycle occurs in the mitochondrial matrix and intermediates of this fundamental metabolic pathway are required for epigenetic processes. Epigenetic processes are those factors that influence gene expression without changing the actual nucleotide sequence of the DNA molecule[62]. One of the best characterised epigenetic processes is DNA methylation, a process key to the regulation of gene expression[63]. Methylated DNA have a covalently bonded methyl group at the carbon-5 position of a deoxycytidine. This is followed by a deoxyguanidine, to form tissue specific methyl patterns[64]. Advancing age has been associated with the disruption of these DNA methylation patterns which are key to the fidelity of gene expression[65]. Specifically, during aging, human DNA undergoes genome wide hypomethylation across a variety of different tissues[66]. Moreover, advancing age also results in regional increases in DNA methylation at the promoter regions of a multitude genes[67]. This alteration, which is referred to as site-specific hypermethylation has significant implications for health[68]. For example, cancers regularly display global hypomethylation and concomitant gene specific hypermethylation[69], while it has also been observed that autoimmune diseases[70] and CVD[71] also manifest this phenomenon.

We derive methyl groups from the B vitamin folate in our diet[72], however deficiencies in the intake of this vitamin or other B vitamins can disrupt the methylation process. However, it has been recently acknowledged that intrinsic aging is also a contributing factor to age-related aberrant DNA methylation[73]. It has been found that with age changes occur to the activity of the enzymes that dynamically regulate DNA methylation patterns[74]. Of these enzymes, DNA methyltransferase 1 (Dnmt1) is primarily responsible for maintaining genomic DNA methylation[75]. DNA methylation events are counterbalanced by active and passive demethylation[76]. Passive demethylation occurs during replication, while active methylation involves ten eleven translocation (TET) dioxygenases, which oxidize the methyl groups of cytosine and appear central to demethylation[77]. Intriguingly, the activity of the TET demethylation enzymes is dependent on fluctuations in α-ketoglutarate an important intermediate in the Krebs cycle[78]. Moreover, several enzymes involved in the Krebs cycle including, isocitrate dehydrogenase, fumarate hydratase and succinate dehydrogenase (SDH) are also known to modulate TET enzymes[78]. Adding further intrigue to the connection between methylation and metabolism, recent experimental evidence has shown that Dnmt1 activity is elevated in response to caloric (CR) in human fibroblast cell lines[79]. Importantly, it has also been suggested that the response of Dnmt1 to CR is mediated by SIRT1, which has been shown to modulate the activity of this key methylation enzyme[80]. Finally, there is also experimental evidence that age related changes to the DNA methylation landscape are at least in part impacted by increases in oxidative stress[81]. For example, it has been shown that DNA lesions, caused by oxidative stress, can disrupt the ability of DNA to function as a substrate for the DNMT1[82]. Taken together, these findings suggest that both ROS emissions by the mitochondria and mitochondrial metabolism could be key players that mediate how DNA methylation changes unfold with age.

Reasons for Adopting Mechanistic Computational Modelling for Aging Research

From our discussion of the aging process, it is apparent that it is an inherently complex process. Traditionally, aging has been investigated like many other aspects of biology in a reductionist manner. However, investigating aging cannot be viewed as just one single aspect of biology. Thus, it is important to acknowledge and appreciate the biological uniqueness of aging and that aging needs to be studied in a holistic manner. Fortunately, there is an increasing appreciation in recent years that biological systems need to be studied within integrated frameworks, and that viewing complex biological systems through a reductionist lens in no longer an adequate experimental paradigm[83]. The aim of systems biology is to provide an integrated understanding of biological processes from the molecular through to the physiological[84]. Computational modelling is an ideal means of facilitating this paradigm shift and they are now increasingly used alongside more conventional biological approaches. The contributions such models can make to the understanding of aging are clear. 1) Computational models can represent the intrinsic complexity associated with aging. 2) Modelling can improve our understanding of the biology underpinning aging and help to generate new insights. 3) It can highlight gaps in current knowledge. 4) A model can help to develop clear, testable predictions about aging that are not always possible to do using conventional means. 5) A model may lead to counterintuitive explanations and unusual predictions about aging that would otherwise be unapparent if the system was not studied in an integrated manner. 6) Models can provide a quick way to analyse a biological system under a wide range of conditions, for example by examining the effects of an array of dietary components. 7) There are many conflicting ideas about aging and models can be used to test a particular hypothesis which may lead to counterintuitive explanations.

APPROACHES TO MODELING AGING

In order to appreciate what computational modeling is, and how it is used in systems biology, it is firstly necessary to give an overview of what it is. Computational modeling is an abstract process which uses mathematics to dynamically represent the components of a biological system and their interactions within a mathematical framework. A key aspect of this techniques is that it allows the simulation of a system's dynamic behaviour. At the heart of computational modeling is mathematics, and there are a number of theoretical frameworks that can be used to construct a computational systems model[85]. The approach that is adopted is largely dependent on the nature of the system that is to be modelled[86]. Recently, Petri nets have been used to model a variety of process in biology[87]. These are a directed bipartite graph, with two types of nodes, called places and transitions, which are represented diagrammatically by circles and rectangles, respectively. Places and transitions are connected via arrows/arcs. Each circle or place contains a number of tokens, which is a kin to a discrete number of biochemical molecules, while the stoichiometry is indicated by the weight above the arrow/arc. Tokens can be both consumed and produced within the Petri net. A Petri net functions by input-output firing at the transitions within the network. The 'firing' of transitions is a kin to a biochemical reaction taking place. Biological systems can also be represented with a Bayesian network (BN)[88]. BNs are a type of probabilistic network graph, where each node within the graph represents a variable. Nodes can be discrete or continuous and are connected to a probability density function, which is dependent on the values of the inputs to the nodes. Agent-

based models have been increasingly used in aging research also[89]. This is a rule-based approach which is used to investigate biological systems using clusters of independent agents whose behaviour is underpinned by simple rules. These agents are capable of interacting with one another through space and time. However, by far the most commonly adopted theoretical approach to modelling in systems biology is a deterministic framework. However, more recent developments have witnessed the adoption of stochastic modelling. In the next sections we will introduce these two important approaches and will highlight some examples that have been used in recent aging research.

Deterministic models versus Stochastic Models

Deterministic models can be represented mathematically by ordinary differential equations (ODEs). ODEs are known as ordinary because they depend on one independent variable (time), and use the assumption that biological species exist in a well-mixed compartment, where concentrations can be viewed as continuous. These systems can be defined as follows

$$\frac{dx}{dt} = f_x(x, y \ldots\ldots t)$$

$$\frac{dy}{dt} = f_y(x, y \ldots\ldots t)$$

x, and y are referred to as state variables, for example these could be the concentration of ROS in a cell, the length of a telomere or the concentration of mTORC1. Species concentration is generally denoted by the state variable enclosed within a square bracket. In the equations f_x, f_y, are the functions describing the molecular interactions. Systems of ODEs that are used to represent biological processes are generally too complex to solve analytically. Therefore, numerical integration is used to simulate their behaviour using a computer. Computational systems biology software tools come equipped with algorithms for doing this, which helps to facilitate the modeling process for those less familiar with mathematics[90].

Continuous deterministic ODEs are based on the assumption that large numbers of molecules are involved in biological reactions and that the random interactions between these molecules has a negligible impact on the behaviour of the system. This makes continuous deterministic models unsuitable for representing process which are governed by stochasticity or randomness within cells. The main sources of stochastic variability at the cellular level are fluctuations in biochemical reactions, which drive a number of processes including gene expression, transduction signalling, and biochemical pathway signalling[91]. These reactions occur through random collisions and transient binding of various molecular species within the cell. This makes these reactions prone to significant noise. In order to deal with this noise stochastic reaction models attempt to represent the discrete random collisions between individual molecules. These type of models treat molecule interactions as random events. A stochastic model is usually underpinned by a propensity function, known as the

Gillespie equation[92]. This equation explicitly gives the probability aμ of a reaction μ occurring in time interval (t, t + dt).

$$a\mu\, dt = h\mu c\mu\, dt$$

The M reactions in the system are given an index value of μ ($1 \leq \mu \leq M$) and hμ implies the number of possible combinations of reactant molecules involved in reaction μ. In essence each reaction within the system has a different probability of occurring. In practice the Gillespie algorithm or one of its variants[92-94] is embedded within a computational modelling tool. Therefore, it is only necessary for the user to have a reasonable understanding of underlying theory of the Gillespie algorithm in order to build a stochastic model of a biological system. At this point it is important to acknowledge that in addition to these approaches, there are a number of other theoretical frameworks that can be employed to model biological systems. These include Petri nets, which are a graphical tool for the description and analysis of concurrent processes[95], Bayesian networks, which are probabilistic graphical models[96], Boolean networks in which entities are either in an on or off state[97], systems of partial differential equations (PDEs), which are multivariable functions that deal with partial derivatives[98] and agent based modelling, which is a rule-based, discrete-event and discrete-time approach that uses objects and rules to simulate interactions among the individual components of the model[99].

Modelling Tools and Model Exchange

A variety of software tools are available for building models and the choice of software tool is dependent on the level of experience of the individual assembling the model. Certain tools are more suitable than others for novice model builders. ODEs can be coded manually by using a commercial software tool such as Matlab or Mathematica. Non-commercial software tools such as Copasi[100] or CellDesigner[101], which have graphical user interfaces, allow the user to build the model by creating a succession of kinetic reactions/a process diagram, which in the in the case of a deterministic model is then converted to a series of coupled ODEs. As discussed in the previous section the software tool then uses an algorithm to solve the ODEs and produce a deterministic output. Once a computational model has been assembled, it is important that it can be both easily accessed and updated by the community as a whole. To facilitate model portability a number of exchange frameworks have been developed[102]. These frameworks allow models to be shared and reused by researchers even if they do not use the same modeling software tool. At present, the leading exchange format is the systems biology markup language (SBML)[103]. This framework is supported by a broad range of modelling software tools (http://sbml.org/SBML_Software_Guide/SBML_Software_Summary). Models that have been encoded in this format can be archived in the BioModels database, a repository designed specifically for archiving models of biological systems[104].

Computational Models of Mitochondrial Dynamics

As outlined, oxidative stress and the emission of ROS by mitochondria is one of the fundamental cellular processes that impacts aging. Therefore, it is unsurprising that various aspects of mitochondrial dynamics have been modelled over the years (for a comprehensive review see Kowald and Klipp (2014)[16]). An early network model of mitochondrial dynamics that examined this was developed by Kowald and Kirkwood (1994). This model showed that during increased free radical production and/or inadequate protection from these free radicals, damage can occur to an otherwise stable translation system[105]. Another area of keen focus is mitochondrial fission and fusion. Briefly, fission and fusion events can be viewed as mitochondrial caretakers whose responsibility it is to control cellular ATP concentration, and to mitigate against the accumulation of damage to mitochondrial DNA (mtDNA). One of the earliest models that focused on these processes was the model developed by Kowald et al. (2005). In this model stochastic simulation of mitochondrial replication, mutation and degradation showed a low mosaic pattern of oxidative phosphorylation (OXPHOS) impaired cells in old organisms[106]. More recently, Tam and colleagues (2013) used computational modelling to investigate the effects of mitochondrial fusion and fission dynamics on mutant mtDNA accumulation[107]. In this stochastic model, simulations indicated that the slowing down of mitochondrial fusion-fission results in higher variability in the mtDNA mutation burden among cells over time, and mtDNA mutations have a higher propensity to clonally expand due to an increase in stochasticity. The model was able to suggest that the protective ability of retrograde signalling (biochemical communication between mitochondria and nucleus) depends on the efficiency of fusion-fission process[107]. Another model which focuses on fusion-fission cycles is the model by Figge and colleagues (2012). This probalistic model demonstrated that cycles of fusion and fission and mitophagy are needed to maintain a high average quality of mitochondria, even under conditions in which random molecular damage is present[108]. Recent mitochondrial models have also focused on specific regions within the mitochondrial ETC. For instance, a model of superoxide production at complexes I and III of the ETC, was able to generate an improved mechanistic understanding of how ROS are generated by complex III. This model also described ROS production by antimycin A inhibited complex III. In order to validate the model, output from its simulations was matched to experimental data from rodents[109]. On a similar theme Markevich and Hoek (2015) used a computational model of mitochondrial bioenergetics to monitor superoxide production under different substrate conditions. Their model suggested that the semiquinone of Complex I should be included as an additional source of ROS[110].

Telomere Models

A number of models have explored telomere dynamics. Most recently, Bartholomäus and colleagues (2014) used a computational model to investigate telomere length under a variety of perturbations[111]. The model was used to explore telomeres during different conformational states, specifically t-loops, G-quadruplex structures and those being elongated by telomerase. This deterministic model was used to examine how different levels of telomerase impacted telomere length. Moreover, the authors used the model to explore the impact of adding different levels of a G4-stabilising drug on the distribution of telomere lengths. Several older models can be found in the literature. Others of note include the model by Rodriguez-Brenes and Peskin (2010) who modelled telomere state on the basis of the biophysics of t-loop formation[112]. The model was able to predict the steady-state length distribution for telomerase positive cells, the time evolution of telomere length, and the life span of a cell line on the basis of the levels of telomeric repeat-binding factor 2; a protein that protects telomeres from end-to-end fusion of chromosomes. The model was also able to predict the life span of a cell line based on telomerase levels. Stochastic models of telomere dynamics include the model by Portugal et al. (2008) which made the assumption that cell division is a stochastic phenomenon whose probability decreases linearly with telomere shortening[113]. Proctor and Kirkwood (2003) also used a model informed by probability to model cellular senescence as a result of telomere state[17]. From an oxidative stress perspective Trusina (2014) recently used a computational model to investigate the effect of genotoxic stress on telomere attrition[114]. Virtual populations of cells were compared and it was found that when ROS was distributed unequally among cells, telomere shortening increased longevity, while also reducing the DNA mutation rate.

Computational Modelling of Metabolic Signalling

In the first section of this paper we described the increasing attention there has been on certain metabolic pathways and how they may have a significant role to play in longevity. Most notably we identified those metabolic pathways that are defined by mTOR and by sirtuins. Several attempts have been made to computationally model various aspects of these pathways[115]. For example, Kriete et al. (2010) developed a computational model that included the mTOR pathway together with other pathways associated with intrinsic aging[116]. This rule based model is of note as it encapsulated many important aspects of aging, including mitochondrial biosynthesis, metabolic fluxes, mTOR as an energy sensor and NF-κB, to detect oxidative stress. Another model which successfully included oxidative stress is that developed by Smith and Shanley (2013)[117]. By building a model of insulin signalling in rodent adipocytes that included transcriptional feedback through the Forkhead box type O (FOXO) transcription factor, it was demonstrated that oxidative stress can have a significant effect on insulin signalling and aging. The model produced a range of findings including the combination of insulin and oxidative stress produced a lower degree of activation of insulin signalling than insulin alone. Antioxidant defences were upregulated in the presence of fasting and weak oxidative stress, whereas, stronger oxidative stress caused short term activation of insulin signalling. The model also demonstrated that if prolonged high insulin may negate the protective effects of moderate oxidative stress. The complex nature of this model is evident, but, combining it with other factors that can influence insulin signalling such as the mTOR pathway could add to our understanding of insulin signalling.

Computational Models of DNA Methylation Dynamics and Aging

In spite of increasing age related experimental data there is a paucity of computational models that have focused specifically on intrinsic aging and DNA methylation dynamics. However, methylation dynamics have been represented computationally within a number of disease states. For instance, Mc Govern et al. (2012) developed a dynamic multi-compartmental model of DNA methylation, which was used as a predictive tool for hematological malignancies[118]. The model centred on the activity of DNMTs. PDEs were used to represent methylation reactions and the model was able to predict the relative abundances of unmethylated, hemimethylated, fully methylated, and hydroxymethylated CpG dyads in the DNA of cells with fully functional Dnmt and Tet proteins. It would be worthwhile adapting this model to include oxidative stress, folate biochemistry and the effects of aging on the activity of the methylation enzymes. This model is also deterministic in nature. However, it has been recognised that DNA methylation dynamics are susceptible to inherent stochasticity[119]. Consequently a number of theoretical frameworks have been proposed for modeling the noise associated with DNA methylation dynamics. For example, reduced mathematical representations of methylation dynamics have been proposed by Riggs and Xiong (2004)[120] and more recently by Jeltsch and Jurkowska (2014), in which DNA methylation at each genomic site is determined by the activity of Dnmts, demethylation enzymes, and the DNA replication rate[121]. An awareness of the stochastic nature of these mechanisms has important implications for the aging process, as experimental evidence indicates that the persistent nature of the human methylome results give rise to this noise[122]. Accordingly, it is imperative that computational models which seek to represent the dynamics of DNA methylation need to account for this inherent variability. One such recent model that has dealt with the intrinsic stochasticity associated with DNA methylation is the model developed by Przybilla et al. (2014), which simulated age-related changes of DNA methylation in stem cells. The findings of this model, which compared age-related changes of regulatory states in quiescent stem cells, with those observed in proliferating cells, suggest that epigenetic aging strongly affects stem cell heterogeneity and that homing at stem cell niches retarded epigenetic aging[123].

Cholesterol Metabolism and Aging

The aging process results in the gradual decline of a biological system. This decline is associated with a broad range of pathological states. An example of this decline is the dysregulation of cholesterol metabolism which is inextricably linked to CVD. Therefore, a keen area of focus is how intrinsic aging impacts whole-body cholesterol metabolism[124-127]. Recently we developed a whole-body model that attempted to capture whole-body cholesterol metabolism. The model was used to examine how age related mechanistic changes to the intestinal absorption of cholesterol resulted in a rise in low-density lipoprotein cholesterol (LDL-C), as increased levels are a risk factor for CVD. The model also revealed that an age related decrease in the hepatic clearance of LDL-C resulted in significant rise in LDL-C by 65 years of age. This model is coded in SBML and is archived in the BioModels database (http://www.ebi.ac.uk/biomodels-main/BIOMD0000000434). In theory this model should be straightforward to update and expand to include other important aspects of aging. As we have eluded to, the free radical theory of aging is a useful means of gluing together disparate aspects of the aging process. It is therefore possible to extend this model by framing it around the insidious rise

in ROS that occurs with age in endothelial, vascular smooth muscle, and adventitial cells. This rise in ROS is suggested to be the key driver in a signalling cascade that results in atherosclerosis. Atherosclerosis occurs when LDL molecules migrate into the artery wall at a site which is undermined by endothelial damage. The LDLs are then oxidised upon coming into contact with ROS. The oxidatively modified lipoproteins (oxLDL) are more atherogenic than the native LDL and lead to the recruitment of the macrophages to the site of the lesion. Monocytes pass into the intima before differentiating into macrophages. These molecules engulf oxidized LDL to form cholesterol-laden foam cells. This ultimately results in the formation of an atherosclerotic plaque which eventually ruptures and causes an artery to block[128] (Figure 2). This can lead to a stroke or myocardial infarction[129]. Computational modeling offers a way of dealing with the different molecular, cellular and hemodynamic events associated with this process.

Brain Aging and Pathology

Recently, we also created a computational model which incorporated key brain regions that characterise AD and combined these with the homeostatic regulation of the stress hormone cortisol[130]. The aim of this model was to examine how increased levels of cortisol impinge on the integrity of the hippocampal region of the brain, which is the core pathological substrate for AD. The model was able to replicate the *in vivo* aging of the hippocampus. Moreover, both acute and chronic elevations in cortisol increased aging-associated hippocampal atrophy and concomitant loss in the activity of the hippocampus. This computational systems model could be updated to include a number of other processes. For instance, cortisol is synthesised from cholesterol and also acts is also involved in provoking the breakdown of lipids, and a wide variety of other metabolites[131]. Therefore, the model could be integrated with the cholesterol model discussed previously. Moreover, this model could be used as a framework for investigating vascular dementia (VAD). VAD is underpinned by a dysregulation in the supply of O_2 following a stroke or small vessel deterioration, and oxidative stress is central to the processes that underpin the progression of VAD[132]. Oxygen deprivation results in mitochondrial dysregulation and the release of ROS[133]. This increase in oxidative stress damages blood vessels and neurons, resulting in a process which has been termed neurovascular uncoupling[134, 135]. Moreover, this burst of ROS can disrupt mitochondrial function and further induce hypoxia and oxidative stress[136].

A recent ODE model explored a number of the cellular processes associated with Parkinsons Disease (PD). Among the many cellular features of this model, the feedback interactions between damaged α-synuclein and ROS[137] were explored. Simulation results showed, hat the Parkinsonian condition, with elevated oxidative stress and misfolded α-synuclein accumulation, can be induced in the model by intrinsic aging, together with exposure to toxins and genetic defects. Computational approaches could also be used to investigate other key aspects of brain aging. For instance, many individuals with Parkinson's disease report problems with their respiratory, cardiovascular, and gastrointestinal systems[138]. There is also ample evidence of increased neuroinflammatioin Parkinsons individuals, due to oxidative stress, with reports of increased levels of cytokines, macrophages and microglia activation in brain tissues[139,140]. A computational model could thus consider abnormalities in central autonomic nuclei, as to our knowledge, there have been no studies to determine whether abnormalities in central autonomic nuclei contributes to autonomic dysfunction or whether

peripheral autonomic nuclei also show perturbed development and increased inflammation in PD. Autonomic dysfunction could be reflective of systemic autonomic pathology in PD, and that in fact PD is, in part, an autonomic disorder. It is therefore logical that integrated approaches are required to disentangle the pathological onset of this disease. A worthwhile approach that could address these questions would be to construct a computational systems model of these key processes. In Figure 3, we have used Systems Biology Graphical Notation (SBGN) to represent these processes, which could be modelled computationally.

Other recent Models that have focused on Integrating Aspects of Aging

To date, no model has been able to represent aging in its entirety. However, there have been a number of recent examples, whereby various components associated with aging have been integrated together within a mathematical framework, in an attempt to complete a more global view of how aging impacts a particular biological system. For example, Xue and colleagues (2007) demonstrated that aging is associated with the alteration of a few key brain network modules instead of many, and that the aging process preferentially affects regulatory nodes involved in network stability[141]. Multi-level aging based models have also been used to gain an insight into intracellular protein aggregate damage, during aging in *Escherichia coli*[142]. Moreover, multi-scale models have also had a mammalian focus, for example to examine collagen turnover and the adaptive nature of arterial tissue, in response to mechanical and chemical stimuli[143]. Furthermore, this type of modelling has also been utilised to examine disease pathophysiology, such as the muscle fibre arrangement and damage susceptibility in Duchenne muscular dystrophy[144].

FUTURE OPPORTUNITIES AND CHALLENGES

As outlined, the intrinsic biological mechanisms which characterise the aging process are complex and their activities transcend scale and time. In addition, they involve the interplay of a broad range of molecular, biochemical and physiological processes. In the main, computational models have focused on these process at a cellular level. However, these models are not an adequate representation of whole body human aging. In the final section, we will explore the challenges and opportunities for the future integration of mechanistic models associated with the aging process.

Embedding Existing Models into a Multi-Scale Holistic Framework of Aging

A long term goal of aging research is to have whole-body mechanistic models of the aging process. It is important to note that there are currently no models of this nature in existence. However, in order to fully computationally represent aging from cell to tissue level, there are a number of outstanding challenges that remain. Rather than reinventing the wheel it is worth considering extending existing models. In this final section we will outline some of the challenges that exist in combining models and will propose a number of potential solutions. It is important to recognise that a number of these

biological systems need to be further characterised before they can be successfully represented by a computational model. A solution to this problem could be to firstly work on aspects of the aging process that are reasonably well characterised, so that future models are founded upon well characterised biological mechanisms. Moreover, it is important that model building is coupled closely with wet-laboratory experimentation. Systems biology experiments that are designed with existing *in silico* models firmly embedded within their methodology would significantly improve both the model and extend our understanding of the underlying biology. Another significant issue relates to representing biological systems at different levels of scale. It is common place to represent biological systems using models which consist of a system of ODEs that can be analysed, whose dynamics can be solved using a computer. This deterministic approach neglects those reactions that occur at a much smaller scale and involve fluctuations in low molecular populations. Implementing models which combine both the deterministic and stochastic features of biological systems is challenging. However, recently there have been some examples of computational models that have succeeded in accounting for both these effects. For example, Singhania (2011)[145] used a hybrid approach that combined differential equations and discrete Boolean networks to represent mammalian cell cycle regulation. This is particularly important from the perspective of the aging process as in order to truly represent it requires the integration of a variety of processes which traverse different biological and temporal scales. Assembling holistic models which represent the aging process is also hindered by the need to determine realistic values for the many parameters that are the essence of large complex models of biological systems. Due to the nature of the experiments it can be difficult to estimate these parameters from existing experimental data. It is important to recognise however that this is a persistent problem within systems biology generally. Thus, as previously eluded to it is necessary to align computational modelling within any future experimental methodology. In addition a broad range of statistical techniques have been applied to this area recently. For instance, Aitken et al. (2015) embedded an algorithm based on Bayesian inference within the computational systems biology software tool Dizzy[146, 147]. There are several other approaches in which statistical techniques can be used to estimate unknown parameters in systems biology[148]. Continuing developments in this area will no doubt increase in the utility of computational systems models, and this will be of benefit to those models which represent aging.

Conclusion

In this paper we have presented a broad overview of some of the processes associated with the biology of aging. We have also introduced a number of approaches that are currently used to computationally model biological systems and have described in detail a number of models that have been developed to represent a wide variety of discrete components of the aging process. Some of these models include the key role of ROS in the aging process, while others do not. From our perspective, it is hoped that by converging around ROS in coming years we will witness a more comprehensive view of aging that encapsulates the various different mechanisms and their interactions, whose dysregulation result in age associated disease.

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Figure Legends

FIGURE 1. An integrated overview of aging and some of its key players. This figure emphasises the extent of interplay between the different components that underpin intrinsic aging, and how age-related changes to these components affect health-span and longevity. The integrated nature of this diagram highlights the complexities of ageing and why computational models are needed to help

study its dynamics. IGF-1, insulin-like growth factor-1; ROS, reactive oxygen species; PARP, poly ADP ribose polymerase; mTOR, mammalian target of rapamycin.

FIGURE 2. Integrating a computational model of cholesterol metabolism with a variety of other factors involved in the onset of CVD. Our extended model is framed around the insidious rise in ROS that occurs with age. This rise in ROS is a key driver which underpins a pathological cascade that ultimately results in CVD.

FIGURE 3. An SBGN representation of the autonomic nervous system. The aim of this proposed model would be to simulate physiological responses associated with the autonomic nervous system such as heart rate, rate of movements in the gastrointestinal tract, or synthesis of B cells by the spleen. These processes are regulated in part by neurotransmitters and cytokines. Dysregulation of these processes together with oxidative stress have been strongly implicated in the pathology which underpins Parkinson's disease. NE, Norepinephrine; 5HT, serotonin; Ach, acetylcholine.

Further Reading

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